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The Measurement of Sexual Selection Using Bateman's Principles: An Experimental Test in the Sex-Role-Reversed Pipefish *Syngnathus typhle*[1]

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SYNOPSIS. Angus J. Bateman's classic study of sexual selection in *Drosophila melanogaster* has had a major influence on the development of sexual selection theory. In some ways, Bateman's study has served a catalytic role by stimulating debate on sex roles, sexual conflict and other topics in sexual selection. However, there is still considerable disagreement regarding whether or not ''Bateman's principles'' are helpful in the study of sexual selection. Here, we test the idea that Bateman's principles provide the basis for a useful method to quantify and compare mating systems. In this study, we focus on the sex-role-reversed pipefish *Syngnathus typhle* as a model system to study the measurement of sexual selection. We set up artificial breeding assemblages of pipefish in the laboratory and used microsatellite markers to resolve parentage. Three different sex-ratio treatments (female-biased, even and male-biased) were used to manipulate the expected intensity of sexual selection. Measures of the mating system based on Bateman's principles were calculated and compared to the expected changes in the intensity of sexual selection. We also compare the results of this study to the results of a similar study of Bateman's principles in the rough-skinned newt, a species with conventional sex roles. The results of this experiment show that measures of the mating system based on Bateman's principles do accurately capture the relative intensities of sexual selection in the different treatments and species. Thus, widespread use of Bateman's principles to quantify mating systems in nature would facilitate comparative studies of sexual selection and mating system evolution.

INTRODUCTION

Mating system evolution is a central topic in the study of sexual selection. In light of the myriad studies that have used molecular markers to characterize patterns of mating in natural populations (Avise *et al.*, 2002; Griffith *et al.*, 2002), it now seems clear that the genetic mating system is intimately tied to the sexual selection process. The genetic mating system can be defined as the distribution of biological parentage in a population. Thus, a complete study of parentage, which matches all offspring in a population to their parents, would constitute a complete description of the genetic mating system. This definition intentionally ignores social interactions, territoriality, and other ecological characteristics that are often included in the broader usage of the term ''mating system'' (Emlen and Oring, 1977). The reason for this is obvious: The Darwinian fitness consequences of sexual selection are most directly assessed by the actual reproductive success and mating success of individuals, and these measures of fitness can be extracted directly from a description of the genetic mating system (Arnold and Duvall, 1994). One major remaining question is how best to quantitatively describe genetic mating systems for the study of sexual selection.

A. J. Bateman's (1948) classic study of genetic parentage and sexual selection in *Drosophila melanogaster* provided insights into genetic mating systems that have inspired a major school of thought on the measurement of sexual selection in natural populations. Bateman conducted the first major study of genetic parentage in experimental populations by taking advantage of single-locus mutations with visible phenotypic effects. Thus, the presence of these visible markers in offspring allowed him to ascribe progeny to their true genetic parents. Bateman set up small breeding populations of *Drosophila* in the laboratory, allowed them to mate and assigned parentage to the resultant offspring using the visible genetic markers at his disposal. In the 1940s, such an experiment could be conducted only by using a model organism with appropriate genetic tools. Today, this type of experiment can in principle be conducted on essentially any organism through the use of molecular genetic markers such as microsatellites (Jones *et al.*, 2000*a*; Jones and Ardren, 2003).

The most important conclusions from Bateman's experiments have come to be known as ''Bateman's principles.'' In the vein of Stevan Arnold's (1994) treatment of this topic, we recognize three principles directly attributable to Bateman. The first is that males exhibit higher variance in number of offspring than females; the second is that males have higher variance in number of mates than females; and the third is that males have a higher correlation between number of mates and number of offspring than females. Bateman saw the first two principles as signs of intra-masculine sexual selection and he saw the third principle as the cause of sexual selection on males (Bateman, 1948). In the late 1970s and early 1980s, Michael Wade and

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Steve Arnold focused on the first two principles as a measure of the upper limit on the strength of sexual selection (Wade, 1979; Wade and Arnold, 1980; Arnold and Wade, 1984). It can be shown that the variance in relative fitness is proportional to the maximum strength of selection (Crow, 1958). Because sexual selection involves competition for access to mates (Darwin, 1871; Andersson, 1994), the fitness currency of sexual selection can be viewed as either number of offspring (reproductive success) or number of mates (mating success) within a selection episode (*e.g.*, a breeding season).

In the 1990s, Steve Arnold and David Duvall (1994) seized upon Bateman's third principle and formalized it into a measure of sexual selection with explicit connections to selection theory. Bateman saw this third principle as the cause of sexual selection. The logic was that males have a higher correlation between mating success and reproductive success because their reproduction is limited by access to mates. Females, on the other hand, are limited by their intrinsic reproductive capacity, because a single mating will usually provide enough sperm to fertilize all of a female's eggs (Bateman, 1948). Thus, sexual selection should be stronger on males due to the greater potential payoff males derive by mating with multiple females. Arnold and Duvall (1994) suggest that this relationship should be quantified with a simple linear regression, called the sexual selection gradient. This relationship has more recently come to be known as the Bateman gradient (Andersson and Iwasa, 1996). A steep Bateman gradient results in persistent directional selection on mating success. Under such circumstances, any trait correlated with mating success (such as a secondary sexual character) will be under strong selection. However, if the regression of reproductive success on mating success has a slope of zero, then there is no benefit in terms of offspring production to multiple mating, which means that there will be no selection for traits related to mating success and hence no sexual selection (Arnold and Duvall, 1994).

Bateman's principles, as originally posed in 1948, were narrowly focused on sexual selection in *Drosophila* males, but their modern versions can be generalized and applied to either sex and other species. Based on the first two principles, we expect the sex experiencing more intense sexual selection to exhibit greater standardized variances in reproductive success and mating success. Given the third principle, we expect the sex experiencing more intense sexual selection to have a steeper slope to the linear regression of reproductive success on mating success. Each of these measures of the mating system places an upper limit on the intensity of sexual selection. A complete lack of variance in either mating success or reproductive success precludes sexual selection because there is no variance in fitness in such a system. A zero slope to the Bateman gradient similarly constrains sexual selection, because in such a system there is no fitness benefit to success in mating competition. One important feature of these modern versions of Bateman's principles is that they do not presuppose sexual selection on either sex. Thus, they allow for sexual selection on females, and they also permit sexual selection to act simultaneously on both sexes.

Our goal was to conduct a key test of Bateman's principles by quantifying the genetic mating system in experimental populations of a sex-role-reversed pipefish, *Syngnathus typhle*. In this species, males provide all parental care. The female transfers unfertilized eggs to a pouch on the ventral surface of the male, and the male releases sperm into the pouch to fertilize the eggs. The pregnant male then gestates the offspring for a period of approximately 30 to 40 days. In natural populations, the males are a limiting resource for female reproduction, because the potential reproductive rates of females are higher than those of males (Berglund and Rosenqvist, 1990; Vincent *et al.*, 1994). The direction of sexual selection is consequently reversed relative to most species, because females of this species compete for access to males and males exercise mate choice (Berglund *et al.*, 1986). With respect to Bateman's principles, we expect higher standardized variances in mating success and reproductive success in females than in males. We also expect females to exhibit a steeper Bateman gradient than males, and this prediction has been supported by a more limited analysis of the experiments that we describe here (Jones *et al.*, 2000*a*).

In the present study, we manipulate the primary sex ratio in different breeding trials, because the operational sex ratio is thought to be a major factor determining the intensity of sexual selection (Emlen and Oring, 1977; Andersson, 1994). Because our experiments involve only reproductively mature individuals, manipulation of the primary sex ratio results in a direct change in the operational sex ratio, as defined by Emlen and Oring (1977). Numerous theoretical and empirical studies over the last several decades have demonstrated that the operational sex ratio should indeed be expected to change the intensity of sexual selection, because it is the most immediate determinant of the extent to which the limiting sex will be in short supply in the population (Emlen and Oring, 1977; Andersson, 1994). By assessing patterns of parentage in replicated experimental breeding populations, we addressed four questions. First, do measures of the mating system based on Bateman's principles accurately capture the reversal of sexual selection that occurs in *S. typhle*? Second, do these measures respond as predicted to changes in the sex ratio? Third, do we see evidence for sexual selection on body size, a trait implicated in mate choice in this species? And fourth, how do Bateman's principles from this sex-role-reversed pipefish compare to those derived from a similar experiment conducted using a newt species with conventional sex roles?

MATERIALS AND METHODS

Experimental setup

The pipefish experiments were conducted at Kristineberg Marine Research Station on the Swedish West Coast. Before the onset of pipefish mating activities, in May of 1996, we collected adult male and female *S. typhle* from shallow eelgrass (*Zostera*) meadows in Gullmar Fjord by pulling a small beam trawl (2 mm mesh) behind a boat. Males with empty brood pouches and females in breeding condition move into the shallow eelgrass several days before mating commences, so large numbers of unmated adults suitable for use in breeding experiments can be collected during this period. The artificial breeding populations were set up in an array of 225 liter plastic barrels, each of which was equipped with plastic eelgrass. Temperature, light and salinity regimes followed natural conditions.

This experiment involved three sex-ratio treatments: female excess (6 females: 2 males), even sex ratio (4 females: 4 males) and male excess (2 females: 6 males). Males and females were placed approximately simultaneously in each barrel and were allowed to mate freely for a period of approximately 72 hr. Each sex ratio treatment was replicated nine times, for a total of 27 artificial breeding populations. All fish were then removed from the barrels and measured. Pregnant males were held in smaller tanks for several weeks to allow sufficient brood development for microsatellite-based parentage analysis. Some males failed to mate during the experimental treatments. We placed each of these non-pregnant males in isolation with four females to see if they were capable of mating. If they mated they were retained in the analyses, whereas if they still did not mate we considered them to be incapable of mating (probably due to poor brood pouch condition) and discarded them from further consideration. Some males died or escaped from tanks during the holding period, so those replicates missing a male were removed in their entirety from the analysis. The final data set included 7 replicates of the 6 female: 2 male treatment, 6 of the even sex ratio treatment and 7 of the 2 female: 6 male treatment. At the end of each experimental replicate, the adult females and pregnant males were preserved at -80° C.

Microsatellite-based parentage analysis

Four microsatellite loci, originally cloned from *S. typhle*, were employed for the genetic assessment of parentage. Primer sequences and PCR conditions for the four loci (*typh04, typh12, typh16* and *typh18*) are described elsewhere (Jones *et al.*, 1999*a*). Tissue samples were prepared for PCR by using the Gloor and Engels (1992) extraction protocol as described in Jones and Avise (1997*a*). For pregnant males, we dissected away the outer membrane of the pouch and removed embryos singly with forceps, mapping the position of the embryos as they were removed. We removed and discarded the yolks and placed the embryos individually in microcentrifuge tubes, to which we added 50–150 μl of Gloor and Engels' (1992) fly buffer. We incubated the samples for 30 minutes at 37°C, followed by two minutes at 95°C. After centrifugation at high speed, 2 μl of the supernatant was used as template for PCR. For adults, we used the same extraction protocol, but with small caudal fin clips as the starting tissue sample. PCR fragments were resolved on denaturing polyacrylamide sequencing gels as described in Jones *et al.* (1999*a*).

The successful replicates of the experiment resulted in a total of 76 females, 50 pregnant males, and 15 non-pregnant males that were capable of mating (see above). We determined four-locus microsatellite genotypes of all of the females and pregnant males. We also assayed either every embryo or every third embryo from each male, for a total of 1,131 genotyped offspring. Because offspring are clumped by maternity within the brood pouch and we mapped the locations of embryos within the pouch, this approach resolves maternal contributions to each pregnancy with very little error. Initially, all embryos were genotyped at two loci (*typh04* and *typh16*), and the other two loci (*typh12* and *typh18*) were used to resolve issues of ambiguity. Offspring were matched to mothers by complete exclusion.

Statistical characterization of mating patterns

Measures of the mating system based on Bateman's principles are easily calculated from the genetic parentage analysis. From the molecular results, we determined the number of successful mating events (mating success) and the total number of offspring produced (reproductive success) for each individual. The opportunity for selection (I) is simply the variance in reproductive success divided by the square of mean reproductive success (Wade, 1979; Wade and Arnold, 1980). The opportunity for sexual selection (I_s) is the variance in mating success divided by the square of mean mating success (Wade, 1979; Wade and Arnold, 1980). We calculated these values separately for each artificial population and compared the means among treatments by using analysis of variance. Similar results were obtained by lumping individuals across replicates within a treatment. The Bateman gradient, which embodies Bateman's third principle, is estimated by a simple linear regression of reproductive success on mating success (Arnold and Duvall, 1994). We calculated Bateman gradients for the different sex ratio treatments by pooling individuals across replicates within each treatment (Jones *et al.*, 2000*a*).

We also looked for evidence of sexual selection acting within each treatment on body length, a trait that has been shown to be the target of mate choice in this species (Berglund *et al.*, 1986). We calculated standardized selection differentials (s') on body length in two separate analyses, using either mating success or reproductive success as our measure of fitness. The standardized selection differential is the covariance between the trait, standardized to have a mean of zero and a variance of unity, and relative fitness (Lande and

Arnold, 1983). For treatments with a large number of unmated individuals, we also plotted the histograms of mated *versus* unmated individuals to provide a visual depiction of the phenotypic differences among those individuals that were successful in mating competition *versus* those that were unsuccessful.

RESULTS

Parentage analysis

The microsatellite-based parentage analysis successfully assigned parentage to 1,129 of the 1,131 genotyped offspring. The other two embryos could not be assigned to a female even after being genotyped at all four loci, but they were genetically compatible with the mothers of their neighbors within the pouch, so we assumed that they were the full siblings of the neighboring young. No null alleles were observed, but some germ-line mutations did occur at two of the loci, *typh04* and *typh16,* with mutation rates of 0.003 and 0.0009, respectively. These mutations did not affect the parentage analysis and they are treated at great length elsewhere (Jones *et al.*, 1999*b*).

Effects of the sex ratio on the pipefish mating system

Mating patterns in *S. typhle* changed dramatically in response to variation in the sex ratio. Figure 1 shows mating success histograms for males and females in the three treatments. In the female-excess treatment, which included 6 females and 2 males in each mating barrel, successful females mated with one to three males and more than half of the females failed to obtain mates (Fig. 1). Males in this treatment, on the other hand, enjoyed higher mean mating success (Table 1) and very few males failed to mate (Fig. 1). Thus, male mating patterns were significantly different than female mating patterns (Fig. 1). In the even sex ratio treatment, which included four males and four females per barrel, the males and females displayed very similar patterns of mating success to each other, and indeed the histograms were statistically indistinguishable (Fig. 1). In the male-excess treatment, which included six males and two females per barrel, the distributions of male mating success is again very different than the distribution of female mating success (Fig. 1). Unlike in the other treatments, in this treatment a large proportion of males failed to mate, and as noted above (see materials and methods) these were males that we tested after the mating trials to verify that they were capable of mating. Interestingly, in the male-excess treatment, all females mated successfully, indicating that female competition for mates may have been reduced dramatically by a surplus of brood pouch space (Fig. 1).

Response of Bateman's principles to the sex ratio

The opportunity for selection and the opportunity for sexual selection behaved as predicted in response to changes in the operational sex ratio. We expected sexual selection to be stronger on males than on females in the male-excess treatment, whereas we ex-

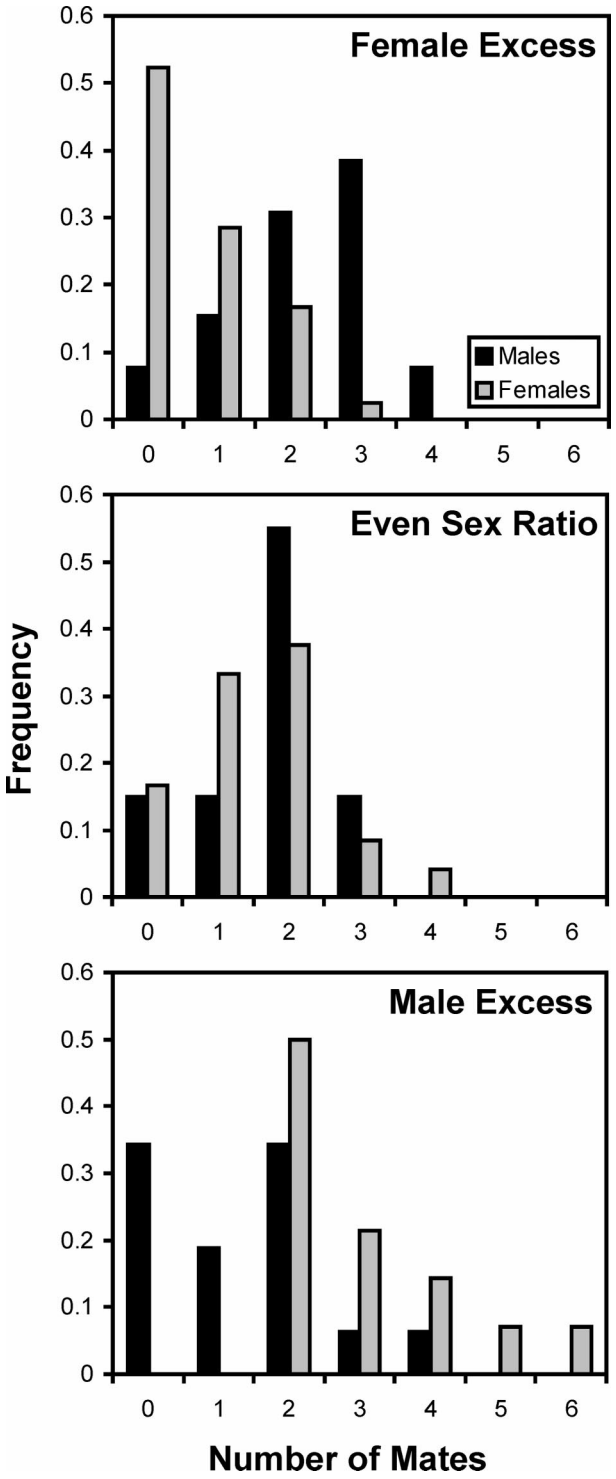

FIG. 1. Mating success histograms for males (black bars) and females (gray bars) in the three sex ratio treatments. In each treatment, male and female distributions were compared statistically to one another using a contingency χ^2 test with ≥ 3 mates lumped into a single category. The male and female histograms differ significantly from one another in the top ($\chi^2 = 21.4$, df = 3, $P < 0.001$) and bottom ($\chi^2 = 13.8$, df = 3, $P = 0.003$) panels, but not the middle panel ($\chi^2 = 2.3$, df = 3, $P = 0.52$).

TABLE 1. *Mating system parameters for males and females in the three sex ratio treatments.**

Sex	$\bar{x}_{MS}$	σ^2_{MS}	$\bar{x}_{RS}$	σ^2_{RS}	S'_{MS} (*P*-value)	S'_{RS} (*P*-value)
Female-Excess:						
Males	2.2	1.2	58.4	1,286.9	0.05 (0.71)	**0.37 (0.02)**
Females	0.7	0.7	18.1	702.3	0.04 (0.84)	0.23 (0.30)
Even Sex Ratio:						
Males	1.7	0.9	41.0	1,162.4	0.14 (0.23)	**0.51 (0.002)**
Females	1.5	1.0	33.5	733.4	0.25 (0.07)	**0.38 (0.01)**
Male-Excess:						
Males	1.3	1.5	30.8	1,181.0	**0.32 (0.04)**	**0.60 (0.001)**
Females	3.0	1.7	70.3	941.1	−0.03 (0.82)	**0.22 (0.04)**

* Shown are mean mating success ($\bar{x}_{MS}$), variance in mating success (σ^2_{MS}), mean reproductive success ($\bar{x}_{RS}$), and variance in reproductive success (σ^2_{RS}). We also present the standardized selection differentials on body length, using either mating success (S'_{MS}) or reproductive success (S'_{RS}) as our measure of fitness. Selection differentials significantly different from zero at $\alpha = 0.05$ are indicated by boldface type. The values shown in this table were calculated by pooling individuals across replicates within a treatment.

pected sexual selection to be strongest on females in the female-excess treatment. The results (Fig. 2) show that, in the male-excess treatment, the opportunity for sexual selection on males is significantly larger than the opportunity for sexual selection on females (Wilcoxon rank sum test, $P = 0.002$). The same pattern is apparent for the opportunity for selection (Wilcoxon rank sum test, $P = 0.002$). In the female-excess treatment, both the opportunity for sexual selection and the opportunity for selection are higher in females than in males (Fig. 2; Wilcoxon rank sum tests, $P = 0.018$ and $P = 0.008$, respectively). In the even sex ratio treatment, I_s and I were not significantly different between the sexes (Wilcoxon rank sum tests, $P = 0.75$ and $P = 0.69$, respectively).

The Bateman gradient also shows interesting behavior in response to the different sex ratio treatments (Fig. 3). In the female-excess treatment, the Bateman gradient for females is significantly greater than zero ($P < 0.001$), but the Bateman gradient for males is not ($P = 0.11$). Similarly, in the even sex ratio treatment, the females exhibit a Bateman gradient that is again significantly different from zero ($P < 0.001$), whereas the male Bateman gradient is positive but not statistically distinguishable from zero ($P = 0.09$). Thus, in the female-excess and even sex ratio treatments, we see essentially identical patterns, with a trend for the Bateman gradient to be steeper in females than in males. When these treatments are analyzed separately, in neither case is the female gradient actually significantly greater than the male gradient. However, when they are combined into a single female-biased operational sex ratio treatment as in Jones *et al.* (2000*a*), the female slope is significantly steeper than the male slope (ANCOVA, $P = 0.004$). In the male-excess treatment we see a different pattern of Bateman gradients. Both sexes exhibit Bateman gradients that are significantly positive (males: $P < 0.001$; females: $P = 0.045$), with the male Bateman gradient slightly but not significantly steeper than the female's. These results are consistent with a slightly different analysis of this same experiment conducted by Jones *et al.* (2000*a*), but the exact values differ slightly because Jones *et al.* (2000*a*) included additional individuals that are not included in the data set under consideration in this analysis.

Evidence for sexual selection on body size

Standardized selection differentials on body length are shown for males and females from the three treatments in Table 1. In principle, sexual selection on a trait should be evident when mating success is used as the measure of fitness in such an analysis. If reproductive success is used as the measure of fitness, then a significant selection differential could be the result of either sexual selection or fecundity selection, a form of natural selection. The results of our selection analysis are very different for males and females. First, in males we observed significantly positive selection on body length in all treatments when reproductive success was used as the measure of fitness. This result is due to the well known pattern that male brood pouch size in pipefish increases with body size, resulting in larger broods for larger males (Ahnesjö, 1995; Jones and Avise, 1997*b*). Hence, fecundity selection favors larger males. When we used mating success as our measure of fitness in males, we observed a significant selection differential only in the male-excess treatment. *A priori,* we expected sexual selection on males to be strongest in this treatment, so overall the selection differentials on males appear to recover the expected patterns of sexual selection and fecundity selection. Figure 4 shows the size histograms for mated and unmated males in the male-excess treatment, and indeed the unmated males are smaller than the mated males, a pattern consistent with positive directional sexual selection on male body size.

The selection results for females are less clear. Surprisingly, in no treatment did we observe a significant selection differential on female body length with mating success as our measure of fitness (Table 1). We did observe significant positive selection on female size with respect to reproductive success in the even sex ratio and male-excess treatments (Table 1). These significant selection differentials result from the tendency for larger females to transfer more eggs per mat-

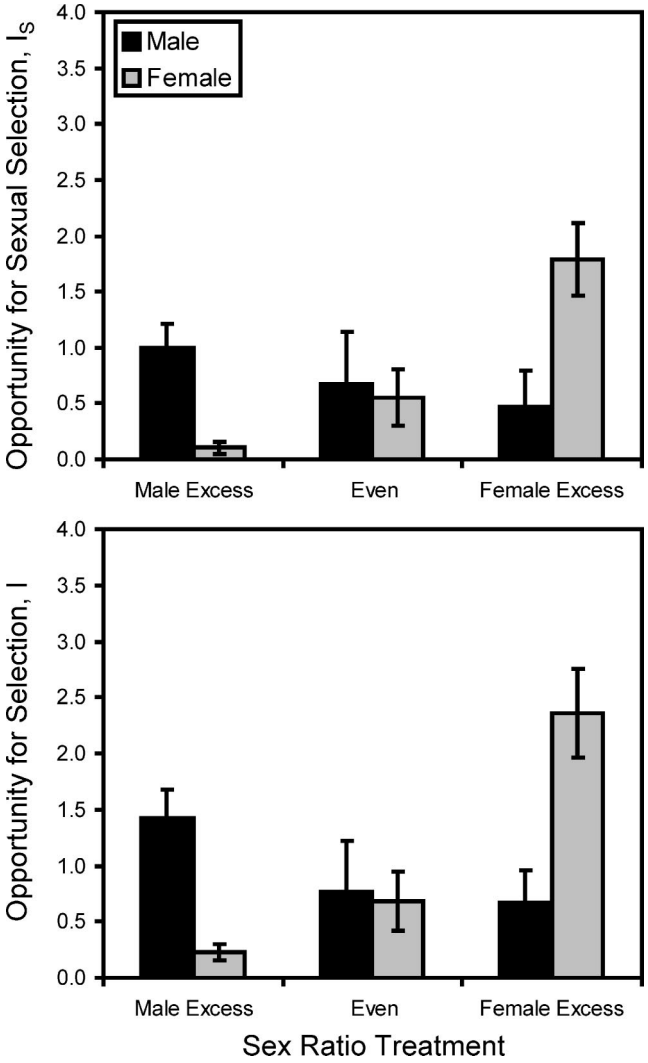

FIG. 2. Opportunities for sexual selection (I_S, top panel) and selection (I, bottom panel) for males (black bars) and females (gray bars) across the three treatments. Error bars show one standard error. Wilcoxon rank sum tests were used to show that males exhibit significantly larger values of I_s and I than females in the male-excess treatment, whereas females show significantly larger values of I_S and I than males in the female-excess treatment (see text for *P*-values). The values of I_S and I did not differ significantly between males and females in the even sex ratio treatment (see text).

ing episode (Berglund *et al.*, 1986; Jones *et al.*, 2000*b*). This pattern of egg transfer could be interpreted as either fecundity selection if larger females transfer more eggs simply because they can produce more eggs or sexual selection if males allow larger females to transfer more eggs per copulation because larger females are preferred (Jones *et al.*, 2000*b*). However, we expected sexual selection to be strongest in the female-excess treatment and did not observe significant selection differentials in this treatment. Another way to address this issue is to look at the size distributions of mated and unmated females in the female-excess treatment (Fig. 4). This analysis shows that the successful females actually tended to be the

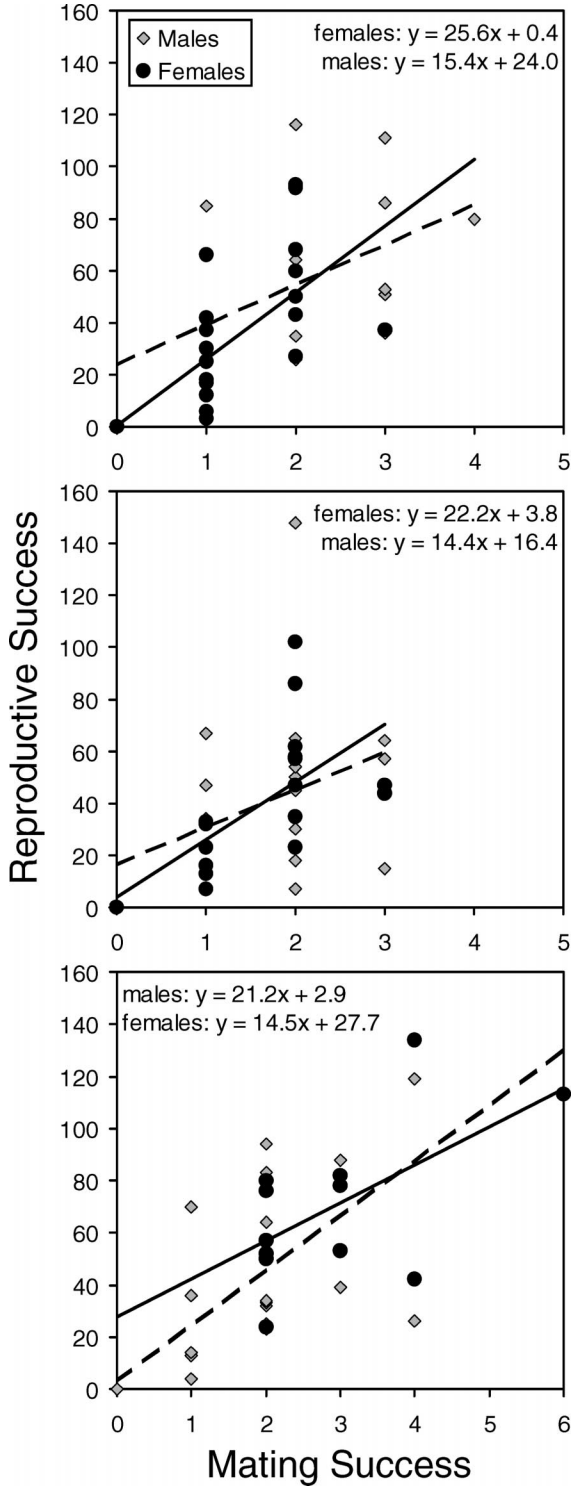

FIG. 3. Bateman gradients for males and females in the three treatments. From top to bottom, the panels represent the female-excess treatment, the even sex ratio treatment, and the male-excess treatment. The Bateman gradients for males are significantly different from zero only in the male-excess treatment (female-excess, $P = 0.11$; even, $P = 0.09$; male-excess, $P < 0.001$), whereas the Bateman gradients for females are significantly different from zero in all three treatments (female-excess, $P < 0.001$; even, $P < 0.001$; male-excess, $P = 0.045$).

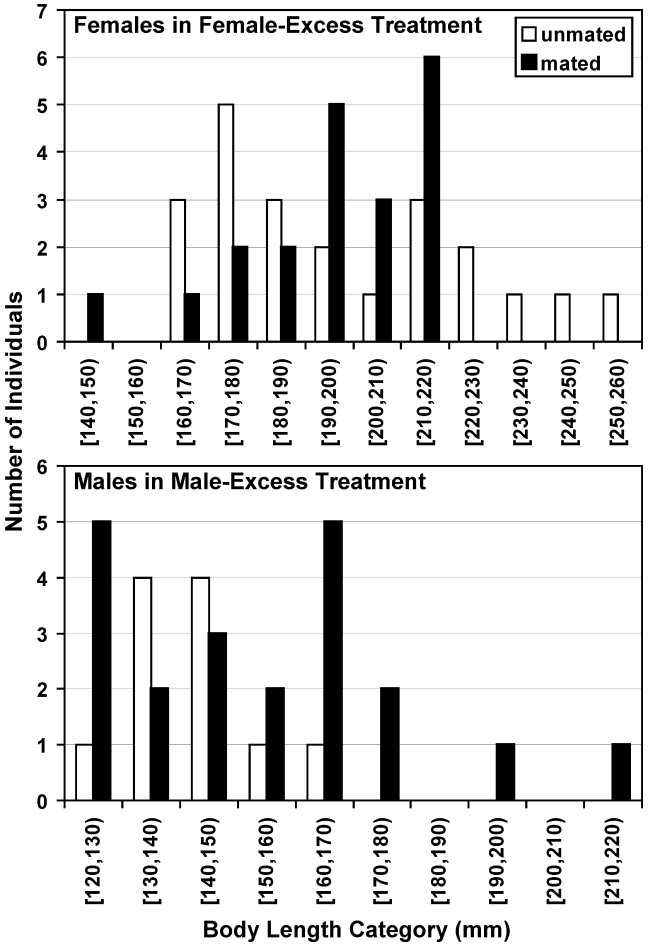

FIG. 4. Distributions of body size for successfully mating and unsuccessful males and females in the treatments for which these distributions were informative. Other treatments had insufficient numbers of unsuccessful individuals for this type of analysis to be useful. The distributions for females (top panel) were compared using a χ^2 test after lumping females into small (140–180 mm), medium (180–220 mm) and large (220–260 mm) size classes. The size distribution for unmated females differed significantly from that for mated females ($\chi^2 = 8.2$, df = 2, $P = 0.02$). For males (bottom panel), larger males enjoyed greater mating success than smaller males, as evidenced by the significant selection differential on male body length (Table 1).

individuals of intermediate size. Several very large females failed to mate, and many of the smaller females also failed to mate. Most of the intermediately sized females actually succeeded in obtaining mates. Thus, there may be stabilizing sexual selection on female body size in this species, a pattern that would not be statistically detectable in the analysis of selection differentials, which can detect only directional selection. A formal analysis of quadratic selection revealed a negative value for γ (-0.17), consistent with stabilizing sexual selection on female body size, but γ was not significantly different from zero ($P = 0.23$). In the male-excess and even sex ratio treatments, we did not have adequately large sample sizes to justify a formal analysis of selection (Kingsolver *et al.*, 2001). Additional research, involving a much larger experiment, will be necessary to determine definitively the true pattern of sexual selection operating on female body size.

DISCUSSION

The results of this study show that the measures of the mating system based on Bateman's principles do respond as predicted to changes in the operational sex ratio. One major tenet of mating system theory is that the operational sex ratio has a major effect on the intensity of sexual selection (Emlen and Oring, 1977; Andersson, 1994). A surplus of males ready to mate is expected to increase the intensity of male-male competition for females, because females will be a limiting resource. Similarly, a surplus of receptive females will promote female-female competition for access to males. The simplest way to manipulate the operational sex ratio is to alter the adult sex ratio, as we have done. The measures of the mating system based on Bateman's principles fulfilled the predictions of mating system theory. The males exhibited higher opportunities for selection (I) and opportunities for sexual selection (I_s) than females in the male-excess treatment. Females showed higher values of I and I_s than males in the female-excess treatment. Interestingly, I and I_s did not differ between the sexes in the even sex ratio treatment.

The Bateman gradients showed a slightly different pattern among treatments than did the variance-based measures. We saw essentially the same pattern in the female-excess and even sex ratio treatments: the gradient for females was significantly steeper than zero but the gradient for males was not. In the male-excess treatment, however, the males' Bateman gradient was slightly steeper than that for females, indicating that sexual selection could be stronger on males in this treatment. Of course, a non-zero sexual selection gradient is necessary for sexual selection to operate. Thus, based on the Bateman gradient analysis alone, we would conclude that strong sexual selection is acting on females in both the female-biased and the even sex ratio treatments. The lack of a significantly positive slope for males in these treatments, however, indicates that sexual selection on males is weak or non-existent. In the male-excess treatment, on the other hand, sexual selection on males appears to be stronger than sexual selection on females, but, because both sexes had significantly positive slopes, sexual selection could be operating on both sexes simultaneously.

The Bateman gradients and variance-based measures of the mating system should be considered simultaneously with respect to their meaning for sexual selection. When we consider the female-excess treatment in relation to the even sex ratio treatment, for example, the Bateman gradient analysis indicates similar intensities of sexual selection in the two treatments. The much higher opportunities for selection and sexual selection in females from the female-excess treatments, however, indicate that sexual selection is potentially much stronger on females in the female-

excess treatment, a pattern consistent with our expectations for this treatment.

Given these considerations, we can summarize the conclusions from the analysis of Bateman's principles. First, in the female-excess treatment sexual selection appears to be acting much more strongly on females than on males, as evidenced by the steeper Bateman gradient and higher values of I and I_s in females. Second, in the even sex ratio treatment, the intensity of sexual selection appears to be higher on females than on males, given the significantly positive Bateman gradient in females. However, the disparity in the intensity of sexual selection between males and females is not as great in this treatment as in the female excess treatment, as evidenced by the similar standardized variances in mating success and reproductive success for the two sexes. Third, in the male-excess treatment, the potential for sexual selection on males appears to be higher than that for females. The male has a slightly steeper Bateman gradient and the opportunities for selection and sexual selection are much higher in males. Across both sexes and all three treatments, the highest potential for sexual selection for females was observed in the female-excess treatment, which also happens to be the treatment that is most similar to conditions in field populations, where we believe that sexual selection usually operates most strongly on females (Vincent *et al.*, 1994).

The major conclusion of this study is that the measures of the mating system based on Bateman's principles correctly recover the changes in the intensity of sexual selection expected from our manipulation of the sex ratio. This result lends additional support to the idea that these measures should serve as the basis for characterizing mating systems in natural populations (Jones *et al.*, 2002). The opportunity for selection and the opportunity for sexual selection were widely criticized after their introduction in the late 1970s and early 1980s (Wade, 1979; Wade and Arnold, 1980). The precise criticisms have been reviewed elsewhere recently (Andersson, 1994; Jones *et al.*, 2002; Shuster and Wade, 2003), so we do not belabor them here, except to say that they fall into two major categories. Some authors felt that variance-based methods were not valid because factors other than sexual selection can contribute to variance in mating success and reproductive success (Clutton-Brock, 1983; Sutherland, 1985*a*, *b*, 1987; Koenig and Albano, 1986; Grafen, 1987, 1988; Hubbell and Johnson, 1987). The second major class of criticism is that the variance-based measures are not the best methods to characterize the outcome of the nonrandom mating process that is a central part of sexual selection (Koenig and Albano, 1986; Grafen, 1987; Sutherland, 1987; Kokko *et al.*, 1999; Fairbairn and Wilby, 2001). Ironically, most of the criticism occurred before sufficiently powerful molecular markers for the routine analysis of parentage had been implemented. The criticisms also mostly predate Arnold and Duvall's (1994) key contribution in which they define the Bateman gradient, and none of them explicitly address the Bateman gradient. Thus, we may be at a turning point in the development of techniques for the characterization of mating systems, and the role of Bateman's principles in this area needs to be reevaluated. Our results indicate that Bateman's principles are useful, and now the question is whether or not this conclusion is general.

Some other recent empirical studies have contributed to the debate about the measurement of sexual selection using Bateman's principles, but ours is one of only two studies that has experimentally manipulated the intensity of sexual selection and asked if these mating system measures can recover the predicted changes in the intensity of sexual selection. A recent study of the rough-skinned newt (Jones *et al.*, 2004) showed that Bateman's principles respond as predicted in response to manipulation of the sex ratio in this species with conventional sex roles. The newt experiment used two sex ratios: a male-excess treatment with eight males and three females per population and an even sex ratio treatment with eight individuals of each sex. The results showed that I and I_s for males were larger in magnitude in the skewed sex ratio treatment than in the even sex ratio treatment (Fig. 5), whereas the I and I_s values for females did not change in response to the sex ratio manipulation. In both treatments, the males exhibited significantly positive Bateman gradients, but the Bateman gradients for females were not significantly different from zero (Fig. 6). Thus, consideration of all three of Bateman's principles indicates that sexual selection was stronger on males than on females in both treatments, and sexual selection on males was stronger in the skewed sex ratio treatment than in the even sex ratio treatment, a result that is perfectly consistent with the expected effects of the sex ratio manipulation. Overall, experiments manipulating the sex ratio in newts and pipefish indicate that mating system measures based on Bateman's principles do an excellent job of capturing the changes in the expected intensity of sexual selection.

Another question related to the performance of Bateman's principles is whether or not a direct assessment of sexual selection on particular phenotypic traits is consistent with the patterns of sexual selection inferred from indirect measures based on the mating system. The results for pipefish lead to some interesting results that are mostly consistent with the idea that sexual selection is acting most strongly in the cases in which I, I_s and the Bateman gradient are all large. In pipefish, we know from behavioral studies that males prefer to mate with larger females and that females prefer to mate with larger males (Berglund *et al.*, 1986). This preference is stronger in males, and this observation contributes to the argument that sexual selection acts more strongly on females in this species. The pattern of sexual selection in the present study is most clear in males. In all treatments, we saw a significant selection differential on male length when reproductive success was used as our measure of fitness. This pattern reflects fecundity selection for larger body

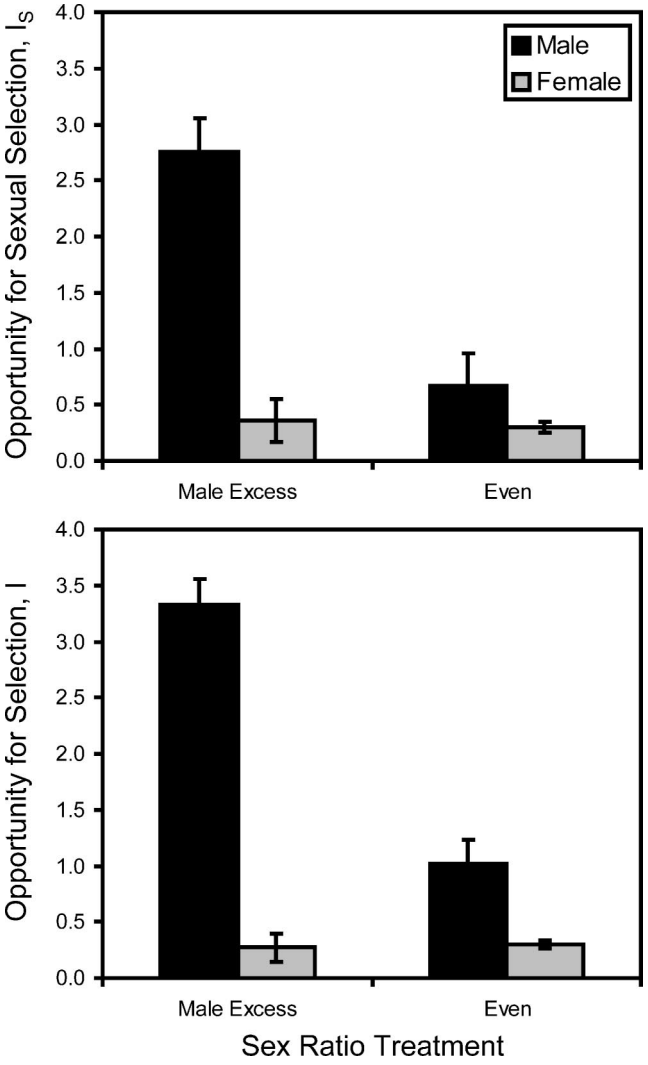

FIG. 5. The opportunities for sexual selection and selection from a similar study of the rough-skinned newt (Jones *et al.*, 2004), a species in which sexual selection typically operates more strongly on males than on females (Jones *et al.*, 2002). The experimental setup was very similar to our pipefish experiment, except that it involved only two treatments, a male-excess treatment and an even sex ratio treatment (see text for more details). Wilcoxon rank sum tests show that I_S is significantly greater in males than females in the male excess treatment ($P = 0.004$) but not in the even sex ratio treatment ($P = 0.42$). The value of I is higher for males in both treatments (male-excess, $P = 0.005$; even, $P = 0.008$).

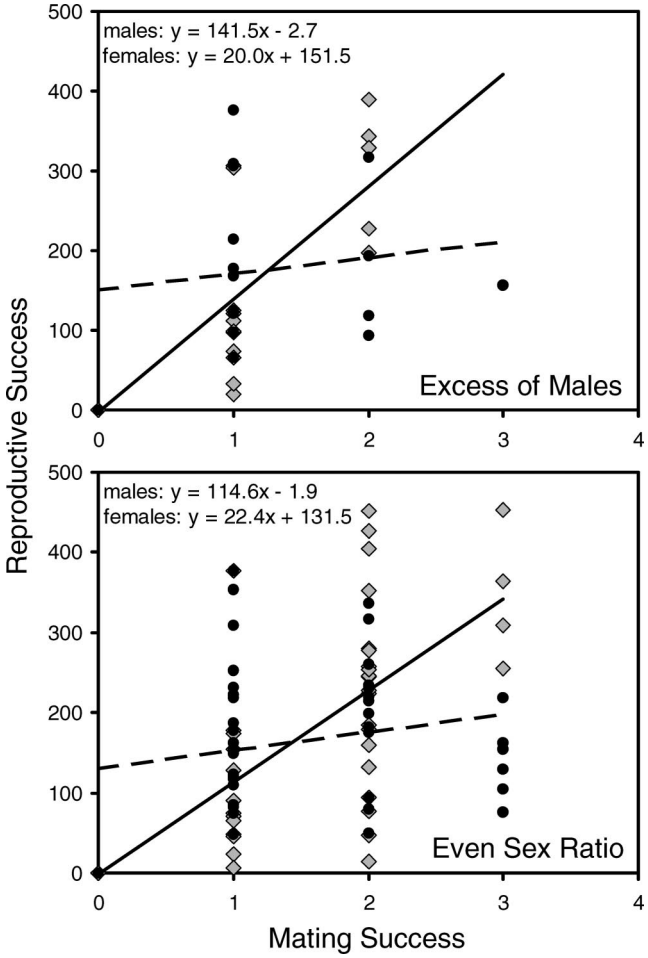

FIG. 6. Bateman gradients from the rough-skinned newt study (reproduced from Jones *et al.*, 2004). In both cases, the Bateman gradients for males are significantly steeper than zero ($P < 0.001$), whereas the Bateman gradients for females are not (male-excess, $P = 0.62$; even, $P = 0.20$).

size, because larger males have larger brood pouches, which can hold greater numbers of embryos (Ahnesjö, 1995). When we used mating success as our measure of fitness, we observed a significant selection differential on male length only in the male-excess treatment, the very treatment in which we expected sexual selection to act most strongly on males.

The patterns of sexual selection on females are less clear. When we used reproductive success as our measure of fitness, we observed significant selection differentials on female body length in the male-excess and even sex ratio treatments only. These significant selection differentials can be explained by the observation that larger females typically transfer more eggs per copulation than smaller females (Berglund *et al.*, 1986; Jones *et al.*, 2000*b*). Unfortunately, we do not know whether this effect is due to male choice or some physical constraint acting on the female. When we used mating success as our measure of fitness, we observed no evidence for a significant selection differential on female size in any treatment. However, inspection of the size distribution of successfully mating females *versus* unsuccessful females in the female-excess treatment shows that the intermediately sized females enjoyed the highest probabilities of reproducing successfully. This pattern of success will produce stabilizing selection on female size, a pattern that will not register in the selection differential. We did not have a large enough sample size in any treatment to detect stabilizing (*i.e.*, quadratic) selection through the techniques developed by Lande and Arnold (1983). These results for females do not eliminate the possibility that sexual selection is acting on females in all three treatments. However, the only treatment in which we saw

a clear statistical signal of non-random mating was the female-excess treatment, in which there appeared to be stabilizing selection on female body size. Overall, the selection results on males and females are consistent with expectations and with the results of the analysis of Bateman's principles. Those treatments in which we expected the greatest intensities of sexual selection on males (*i.e.,* male-excess) and females (female excess) were the only treatments with clear evidence for non-random mating.

One major consideration that needs to be kept in mind during the interpretation of Bateman's principles is that this way of characterizing mating systems in its present form does not accommodate variation in mate or offspring quality. The *prima facie* interpretation of a value of zero for either variance-based measure or the slope of the Bateman gradient is that no sexual selection is operating in the system. The logic underlying this interpretation is that variance in mating success and variance in fitness are both necessary for sexual selection to occur. In addition, success in mating must translate into increased relative fitness for sexual selection to operate, so the Bateman gradient must also be positive. However, this interpretation ignores variance in mate quality and variance in offspring quality. If females choose males on the basis of parenting ability, for example, the benefit to the female may not appear in terms of offspring numbers but in terms of offspring survivorship (Andersson, 1994). Additional studies, beyond the characterization of the genetic mating system, are needed to resolve the role of mate and offspring quality in any particular system.

Given the excellent performance of measures based on Bateman's principles in those studies that have tested them, we believe that a consensus approach to the characterization of genetic mating systems should involve measurement of the opportunities for sexual selection and selection as well as the Bateman gradient. These approaches should also be augmented by an attempt to identify the traits that are the targets of sexual selection and to measure directly the intensity of sexual selection on these traits by using the methods developed by Lande and Arnold (1983). Even in the absence of formal selection analyses, however, Bateman's principles can be useful. For example, they provide a first pass assessment of the intensity of sexual selection that can indicate whether or not sexual selection is likely important in a particular system. These methods also provide a basis for comparison across different systems or populations in which different traits may be the targets of sexual selection. They also permit comparative studies of the potential for sexual selection in species that do not share homologous traits. One other advantage of these techniques is that they provide a composite measure of all sexual selection acting in a system, including unmeasured traits. We do not doubt that some studies will find exceptional cases in which inferences based on Bateman's principles appear to be at odds with the actual intensity of sexual selection, but these cases should be particularly enlightening. Overall, the implementation of Bateman's principles as a widespread technique for the characterization of mating systems should make a major contribution to comparative studies of mating system evolution and the study of sexual selection.

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